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INTRODUCTION

WITHIN the last few years, soybeans have risen from a comparatively obscure place in American agriculture to the rank of a major crop in the central area of the United States. This is illustrated by the following domestic production figures:

Year	Production in Bushels	Year	Production in Bushels
1925	4,875,000	1939	91,272,000
1930	13,471,000	1940	77,374,000
1935	44,378,000	1941	106,712,000

Since soybeans have but recently become an important crop in this country, they have not received the attention of investigators who have studied the factors affecting the respiration of grain in storage.

Respiration is a phenomenon common to all living organisms, but the rate of respiration varies widely as it is affected by both inherent and environmental factors. Aerobic respiration is analagous to oxidative combustion since the initial and final products are the same in both cases. Where hexose sugars are the food materials being utilized, the process may be summed up by the equation:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 677.2 \text{ Cal.}$$

Usually, stored grain is living material, and hence respiration is an inherent characteristic of the grain itself; in addition, the respiration of bacteria, molds, and insects associated with the grain may account for a large share of the respiratory activity exhibited in storage. When respiration occurs at a sufficiently rapid rate to produce heat more quickly than it can be dissipated, the temperature of the grain rises and heat damage may result. Spoilage of stored grain may occur as a result of high respiratory rates even though no temperature rise occurs. Under such condi-

[1] Condensed from a thesis submitted by Paul E. Ramstad to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1942.

A study made in cooperation with the U. S. Regional Soybean Industrial Products Laboratory, a cooperative organization participated in by the Bureau of Agricultural Chemistry and Engineering and the Bureau of Plant Industry of the Agricultural Research Administration of the United States Department of Agriculture and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

tions, much of the damage is due to decomposition caused by the activity of microorganisms. Consequently, a study of the respiratory rates of grain and the factors affecting these rates provides a fundamental approach to the problems involved in grain storage.

In view of the current and prospective economic importance of soybeans, the studies reported here were undertaken to gain information which would assist in laying a sound and scientific basis for methods of grading, shipping, and storing this crop.

HISTORICAL REVIEW

An excellent discussion of the respiratory processes in plants and seeds and a bibliography of the literature in this field has been presented by Miller (36).

Moisture content has long been recognized as one of the major factors determining the intensity of respiration of stored grain. This has been demonstrated with wheat by the researches of Bailey and Gurjar (9) and, later, with corn, oats, barley, rye, and flaxseed by Bailey (7, 8) and with sorghum grains by Coleman, Rothgeb, and Fellows (17). These grains differed not only in their relative rates of respiration at equivalent moisture contents but also in the form of their moisture-respiration curves. Rye, for example, exhibited a low, and flaxseed a high, inherent rate of respiration. The greater respiration of flaxseed was attributed to its high oil content which, being immiscible with water, results in a higher moisture content of the hydrophilic constituents than in cereal grains at equivalent moistures. The accelerating effect of moisture on respiration varied with the different grains; thus, with sound wheat, a sharp break occurred in the curve at 14.5 per cent moisture; whereas, with shelled corn and sorghum grains, there was a fairly uniform increase in respiratory rate with increasing moisture content. In such investigations, the various moisture levels are usually obtained by artificially dampening the grain; the observation of Bailey and Gurjar (9) that the intensity of respiration of wheat increased with time after conditioning when the moisture content exceeded 12 per cent is of particular significance in relation to later researches which revealed the important role played by microorganisms in respiration phenomena at high moisture levels.

Factors other than moisture have a significant influence on grain respiration. Bailey and Gurjar (9) and Bailey (7) have demonstrated that cracked, shriveled, immature kernels respire more rapidly than sound, plump grain of the same moisture content; the presence of foreign material and of sprouted, frosted, or heat-damaged kernels also was shown to increase respiration. In addition, Bailey and Gurjar (9) investigated the influence of temperature and the composition of the atmosphere on the respiration of wheat. Other factors being equal, increases in temperature up to 55° C. caused an increase in respiratory rate. An accumulation of carbon dioxide in the interseed atmosphere depressed respiration; wheat respired in an oxygen-free atmosphere but at a greatly reduced rate.

Microorganisms are always found in abundance in heating grain, but the part they play in the heating and spoilage of stored grain has been very difficult to evaluate. Some investigators have ignored the microorganisms entirely, holding that they take no part in the heating phenomena. Others have believed that microorganisms, and molds in particular, are mainly responsible. The most widely accepted conception today is that both the respiration of the grain and that of the microorganisms are involved, but differences of opinion exist as to the relative importance of these two factors.

Swanson (43) found that, while mold growth is an indication of damage in stored wheat, inhibition of mold growth by exclusion of air or the use of poisons did not prevent injury to wheat quality. An increase in fat acidity was associated with mold growth, but when air was excluded, injury could take place without development of acidity. The moisture content at which molds developed was closely related to the temperature—the lower the temperature, the higher the moisture required for mold growth.

That saprophytic microorganisms growing on dead organic material under the proper conditions of temperature and moisture can respire rapidly enough to cause heating has been demonstrated by Miehe (35), Pierce (37), Darsie, Elliot, and Pierce (19), James (28), and James, Rettger, and Thom (29). However, viable grain is less easily attacked by such microorganisms than dead organic matter. This suggests that studies with nonliving material are not applicable to the case of heating grain; on the other hand, Bakke and Noecker (10), and Robertson, Lute, and Gardner (39) have shown that grain stored under conditions which predispose it to heating loses its viability very rapidly.

Attempts to study the respiration of grain that is free from microorganisms under conditions which would normally favor their growth have been rather unsuccessful. The use of antiseptic agents or treatments strong enough to inhibit or kill bacteria and mold spores is open to the criticism that the respiration of the grain itself may have been affected. Larmour, Clayton, and Wrenshall (32) treated wheat with carbon tetrachloride and found no mold growth or heating even when the grain contained 25 per cent moisture. On the basis of both respiration and heating experiments, these authors concluded that fungi play an important part in the heating of stored wheat, although they pointed out that the treatment with carbon tetrachloride tended to inhibit embryo activity in the wheat, especially at the higher moistures.

Several investigators have made direct observations of grain in transit or commercial storage—Duvel (20), Shanahan, Leighty, and Boerner (40), Duvel and Duval (21, 22). Bailey (5, 6, 8) has pointed out that the prediction of the behavior of stored grain is not a simple matter since its keeping qualities depend upon a number of variables including the mass of the grain, the location, shape, and construction of the storage bin, the initial grain temperature, the inherent characteristics and condition of the grain, and the biological factors involved in respiration.

The soybean (Soja max) is a legume and differs markedly in composition from the grains which have been the subject of respiration and storage investigations to date. Horvath (26) gives the average composition of Manchurian beans as 8.5 per cent moisture, 18 per cent oil, 40 per cent crude protein, 28 per cent nitrogen-free extract, and 5.5 per cent ash. He points out that the composition of American soybeans is similar except that they may contain nearly twice as much moisture. The higher moisture content of American soybeans would indicate that their storage would involve difficulties not encountered with Manchurian beans. Arny, Brookins, and Hodgson (4) have pointed out that improved harvesting methods have been effective in reducing the average moisture content of American grown soybeans. However, unfavorable weather conditions in certain years have resulted in the marketing of soybeans containing excessive moisture.

When the official U. S. standards for the grading of soybeans were established in 1935, the maximum allowable moisture limits for grades Nos. 1, 2, 3, and 4 were 15, 15, 16.5, and 18 per cent, respectively. These moisture limits were based on limited observations of soybeans in commercial storage. On September 1, 1941, the standards were revised to permit 13 per cent moisture in grade No. 1, 14 per cent in No. 2, 16 per cent in No. 3; the maximum for No. 4 remained at 18 per cent.

OUTLINE OF THE INVESTIGATION

The study reported here was undertaken with the purpose of investigating certain of the physical, chemical, and biological factors influencing the relative keeping qualities of soybeans. Only beans of the yellow class were studied since they constitute approximately 90 per cent of the soybeans of commerce.

In view of the importance of moisture in relation to keeping quality, several methods for estimating the moisture content of soybeans were compared, and the hygroscopicity of soybeans and three types of soybean oil meals were determined over a range of relative humidities.

The effects of moisture content, temperature and length of previous storage period, aeration, split beans, and microorganisms on the respiratory activity of soybeans were investigated.

The influence of time and temperature of storage at various moisture levels on the viability of soybeans was determined. Also, the effect of moisture content on certain biochemical changes taking place in soybeans stored in airtight containers for one year at room temperature was investigated.

In order to study directly the heating of soybeans in the laboratory, apparatus was constructed for the adiabatic storage of small quantities of grain. Heating of six bushel lots of soybeans conditioned to various moisture levels was followed by means of a large adiabatic respirometer. In this apparatus, it was not possible to control aeration, so a small scale adiabatic respirometer of one quart capacity, provided with a means for regulating the amount of air passing through the sample, was constructed and used for the simultaneous measurement of heating and respiration.

The laboratory studies were supplemented with observations on soybeans of high moisture content in commercial storage over a two-month period. Temperatures were measured at various depths in the bin, and gas samples taken from these levels were analyzed for oxygen, carbon dioxide, and carbon monoxide.

METHODS OF DETERMINING MOISTURE IN SOYBEANS

The determination of the moisture content of biological materials is empirical, and accordingly it was necessary to standardize the methods to be used for grading purposes. The official basic method for soybeans, as described in "Service and Regulatory Announcement No. 147" (Revised 1939) of the Agricultural

Marketing Service,[2] comprises drying a sample of whole beans to constant weight in a water oven. In actual practice, the Tag-Heppenstall electric moisture meter (a resistance type meter) calibrated against the water-oven method is generally used in the routine grading of soybeans. The method of the American Oil Chemists' Society (2), which is widely used in the oil industry, consists of drying 8 to 10 g. whole beans for three hours in a forced-draft air oven maintained at 130° C.

Cook, Hopkins, and Geddes (18), working with cereal grains, compared the moisture results obtained by different oven methods with those given by the Brown-Duvel method and by several makes of electrical moisture meters. They found that none of the meters tested gave reliable results with grain of high moisture content. Recently, Beckel and Earle (11), in discussing some of the moisture relations of soybeans, have mentioned a number of difficulties encountered in determining their moisture content.

For research purposes, a two-stage vacuum-oven method, similar to that described by Cook, Hopkins, and Geddes (18), appeared to be the most desirable and, unless otherwise specified, all moisture values reported in this study were obtained by this method. Approximately 25 g. soybeans was weighed in a covered weighing vial, transferred to a Petri dish, and allowed to air-dry until in equilibrium with the atmospheric humidity of the laboratory (about two days was required). The sample was reweighed, ground in a Wiley laboratory mill to pass the 1 mm. sieve, and the moisture content of the ground sample determined by drying overnight in a vacuum oven maintained at 100° C. and a pressure not exceeding 25 mm. mercury. From the data thus obtained, the original moisture content was calculated.[3] While this method is tedious and is not adapted to routine work, it is believed to give the most reliable results, particularly with high moisture samples. The preliminary air-drying reduces loss or gain of moisture in the grinding operation; the use of ground rather than whole soybeans reduces sampling error; and drying in vacuo prevents oxidation.

As the moisture method employed here differs from those used in grading and in the oil industries, it was necessary to establish the relations between the results obtained by the three methods in order that the respiration and storage data could be

[2] Since the completion of this study the Official Grain Standards of the United States for soybeans have been amended, making the air-oven procedure (described in Service and Regulatory Announcement No. 147) the official method for determining moisture content, effective September 1, 1942.

[3] Directions for making this calculation are given in U.S.D.A. Service and Regulatory Announcement No. 147, and in Cereal Laboratory Methods (1941) published by the American Association of Cereal Chemists.

Table 1. Comparison of Soybean Moistures Determined by Different Methods

Sample No.	Mean Moisture Content		
	Tag-Heppenstall moisture meter*	A.O.C.S. whole-bean air-oven method	Two-stage 100° C. vacuum-oven method
	per cent	per cent	per cent
1	8.8	9.2	9.6
2	9.1	9.2	9.9
3	9.4	9.6	10.0
4	9.7	10.0	10.4
5	10.0	10.2	10.9
6	10.3	10.2	10.8
7	10.6	10.5	10.8
8	11.0	11.2	11.2
9	11.4	11.9	11.7
10	13.5	14.2	13.7
11	14.2	15.1	14.7
12	14.4	15.0	15.1
13	14.6	15.9	15.9
14	15.0	16.1	16.2
15	13.8	15.0	14.8
16	17.4	19.1	18.8
17	17.0	18.9	18.7
18	16.0	18.0	18.3
19	16.0	17.8	17.7
20	16.2	17.8	18.2
21	16.4	17.9	18.0
22	16.6	18.4	18.3
23	17.2	19.3	19.5
24	17.4	19.9	20.0
Mean	13.58	14.60	14.72
Standard Error (Single Determination)		0.16	0.04
Mean Difference between Duplicates		0.18	0.08

Regression equations:

Tag-Heppenstall moisture = 1.333 + 0.832 Two stage moisture

Whole-bean air-oven moisture = − 0.717 + 1.041 Two stage moisture

* Single determinations.

interpreted in terms of commercial usage. For this purpose, 24 soybean samples, together with their moistures as determined by the Tag-Heppenstall electric moisture meter, were obtained from the Chicago and Minneapolis offices of the Grain and Seed Division of the Agricultural Marketing Service. The moisture contents of these samples were determined in duplicate by the A.O.C.S. air-oven and the two-stage vacuum-oven methods. The mean results are given in table 1, together with the standard errors, mean differences between duplicates, and the regression equations expressing the relationship between the values obtained by the three methods.

These data show that the Tag-Heppenstall moisture meter, calibrated as it is at present, considerably underestimates the moisture, particularly with high moisture samples, that is, in cases where moisture differences become more critical in relation to respiratory activity and storage behavior. While the whole-bean air-oven method gave values averaging only slightly lower than the two-stage vacuum-oven method, the differences between duplicates were much greater, presumably due to a greater sampling error. In view of these results, the two-stage vacuum-oven procedure appears to be preferable to the whole-bean air-oven method in research work.

Most investigators who have studied the effect of moisture content on grain respiration have failed to specify the method they used in determining moisture content. Obviously, in view of the discrepancies in the results obtained by different moisture methods, such data lose much of their quantitative value.

HYGROSCOPICITY OF SOYBEANS AND SOYBEAN OIL MEALS

The hygroscopicity of soybeans is of importance in relation to storage behavior since moisture content not only affects respiratory rate directly, but is also a critical factor with regard to the growth of bacteria and molds. Weather conditions during ripening and harvesting govern the moisture content of soybeans when put into storage and may be responsible for changes in moisture content during storage. Burlison, Van Doren, and Hackleman (14) weighed a crib of stored soybeans at weekly intervals for a period of one year and found that during seasons when relative humidity was highest and temperatures lowest, the beans gained weight, and during the hot, dry summer months they lost weight.

The hygroscopic equilibria of four samples of soybeans representing three varieties were determined by exposing weighed samples of known moisture content at 25° C. in desiccators over sulfuric acid solutions, which were prepared according to the data of Wilson (44) to provide a series of desired relative humidities.

Table 2. Hygroscopic Equilibria of Soybeans at 25° C.

Relative humidity	Moisture Content at Equilibrium*				
	Dunfield 1937	Illini 1938	Scioto 1938	Illini 1939	Mean
per cent	per cent	per cent	per cent	per cent	per cent
35	6.6	6.3	6.7	6.4	6.5
50	8.0	7.9	8.2	8.1	8.0
60	9.5	9.5	9.8	9.5	9.6
70	12.5	12.1	12.8	12.0	12.4
85	....	18.3	18.4	18.5	18.4

* Vacuum-oven method.

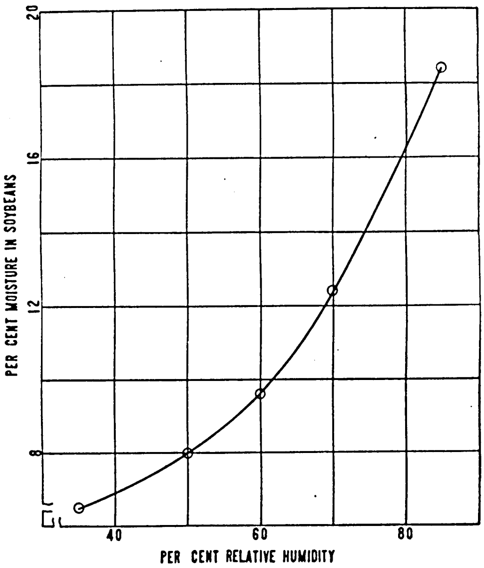

FIG. 1. HYGROSCOPIC MOISTURE OF SOYBEANS IN EQUILIBRIUM WITH ATMOSPHERES OF VARIOUS RELATIVE HUMIDITIES AT 25° C.

The results over the range of 35 per cent to 85 per cent relative humidity are given in table 2. The mean hygroscopicity of the four samples is shown graphically in figure 1.

It is of interest to compare these data with those obtained by Coleman and Fellows (16) with several cereal grains and flaxseed. These workers observed that the hygroscopicity of various cereal grains did not differ greatly but that of flaxseed was much lower, presumably because of its high oil content. Reasoning on the basis of these findings, one would expect that the oil content of soybeans, which averages about one half that of flaxseed, would cause the hygroscopicity of soybeans to lie about halfway between that of flaxseed and the average hygroscopicity of the cereal grains. This is very nearly the case at a relative humidity of 70 per cent. At lower humidities, the hygroscopicity approaches that of flaxseed, and at higher humidities soybeans appear to become more hygroscopic than cereal grains. This comparison is not strictly valid since Coleman and Fellows determined moisture by the water-oven method. However, in view of their high ash content, the hygroscopicity of soybeans would actually be expected to approach that of cereal grains at high humidities when moisture content is determined by comparable methods. Briggs (13) has shown that with increasing vapor pressure the adsorption effect of a colloid becomes relatively insignificant as compared with that resulting from the ions which are bound to it by salt valences.

Large quantities of soybean oil meals are now being produced in the United States as a by-product of the manufacture of soybean oil. The hygroscopicity of these meals is of interest not only because excessive moisture, as a consequence of storage in a

Table 3. Hygroscopic Equilibria of Soybean Oil Meals at 25° C.

Relative humidity	Moisture content at equilibrium*		
	Expeller meal	Solvent meal	Toasted-solvent meal
per cent	per cent	per cent	per cent
10	4.9	5.4	4.8
22	6.3	6.8	6.3
30	7.0	7.5	7.0
40	7.8	8.2	7.8
50	8.9	9.2	8.8
58	10.4	10.8	10.4
69	12.8	13.3	12.8
77	16.9	17.1	16.6
90	24.0†	25.6†	26.5†

* Vacuum-oven method.

† These results are approximate only since no definite equilibrium was attained prior to mold growth.

humid atmosphere, may result in spoilage of the product but also because storing sacks of such meals in a dry atmosphere can cause loss of weight so that they appear to have been underpacked.

The hygroscopicities of three types of soybean oil meals, namely, an expeller meal, a solvent-extracted meal, and a toasted solvent-extracted meal, were determined with the apparatus described by Anker, Geddes, and Bailey (3). The results, recorded in table 3, indicate that the hygroscopicity curves for soybean oil meals are very similar to that for soybeans, although the meals are somewhat more hygroscopic. This would be anticipated because of their lower oil content and, consequently, higher proportion of hydrophilic constituents. Toasting apparently reduced the hydrophilic nature of the solvent meal with the result that it possessed approximately the same hygroscopicity as the expeller meal.

A comparison of these hygroscopicity values with those obtained by Anker, Geddes, and Bailey (3) for wheat flour shows that up to a relative humidity of 70 per cent soybean oil meals are less hygroscopic than wheat flour; above 70 per cent relative humidity they become very much more hygroscopic than wheat flour. In this case, the comparisons are justified since the moisture determinations were made by the same method. The explanation, as already mentioned, probably lies in the high ash content of soybeans. These data indicate that soybean oil meals may show even greater weight changes with variation in atmospheric conditions and may be more prone to spoilage when stored under conditions of high humidity than wheat flour.

RESPIRATION STUDIES AT CONSTANT TEMPERATURE

Methods Used in Respiration Studies

The basic method used for the study of soybean respiration at constant temperature was, with slight modifications, that described by Bailey (8). Briefly, it consisted of determining quantitatively the amount of carbon dioxide produced by a certain weight of soybeans in a given period of time and expressing the respiratory rate in terms of milligrams of carbon dioxide respired per 100 g. of dry matter per 24 hours. In all experiments, except those where temperature was the variable factor, the incubation temperature was 37.8° C. (100° F.). This temperature was used by Bailey and Gurjar (9) in their studies on the respiration of stored wheat and later by Bailey and other workers in studying the respiration of various grains. The temperature of grain before storage is dependent on the temperature of the surrounding atmosphere, and 37.8° C. is very likely to be about the maximum temperature attained before storage.

The apparatus used in these studies is shown in figure 2. Soybean samples were sealed in sheet metal respirometers of approximately 1050 ml. capacity each and equipped with an inlet and an

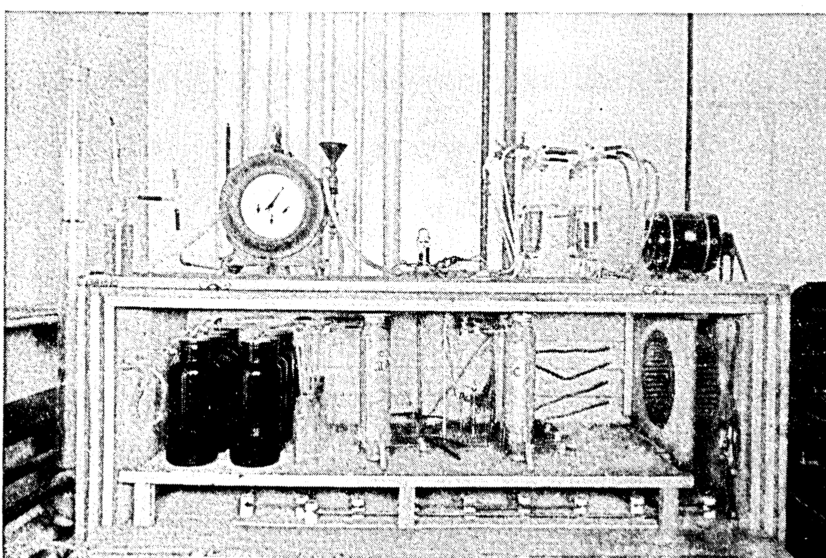

FIG. 2. EQUIPMENT USED IN DETERMINING RESPIRATORY RATES AT CONSTANT TEMPERATURE

outlet tube. The total carbon dioxide respired was determined, usually after four days' incubation, by aspiration through standard barium hydroxide solution in spiral absorbers and back-titration of the residual alkali with standard hydrochloric acid using thymolphthalein as indicator. The aspiration was continued for three hours, and sufficient carbon dioxide-free air was drawn through the grain to replace completely all the air in the system at least four times. The barium hydroxide was 0.0908 *N* (1 ml. equivalent to 2 mg. CO_2), and the hydrochloric acid was one half this strength. Martin and Green (34) have shown that barium hydroxide may be titrated with hydrochloric acid and stirred with air in the presence of barium carbonate without the decomposition of the latter, provided that the strength of the acid is less than 0.07 *N*.

In view of the well known inhibitory effect of carbon dioxide on respiration, an experiment was conducted to determine the effect of different amounts of free air space in the respirometers on the carbon dioxide production of soybeans at two different moisture levels. Changes in amount of air space were produced by varying the amount of soybeans in the respirometers or by adding glass beads. The results presented in table 4 show that even relatively low concentrations of carbon dioxide cause a reduction in respiratory rate.

In order to reduce inhibition of respiration by carbon dioxide accumulation in subsequent experiments, smaller quantities of soybeans were used when they possessed a high rate of respiration than when the soybeans had a low respiratory activity. In a few cases where the samples exhibited very high respiratory activity, it was necessary to reduce the incubation period to two days. An attempt was made to adjust conditions so that the total amount of carbon dioxide produced during the incubation period was less than 50 mg.

Table 4. Effect of Free Air Space on Rate of Respiration*

Moisture content	Air space per gram of soybeans	Final CO_2 concentration	Respiration at 37.8° C. CO_2/100 g. dry matter per 24 hours
per cent	ml.	per cent	mg.
15.1	2.2	2.2	2.3
15.1	3.0	1.6	2.6
15.1	4.4	1.3	2.9
18.6	4.6	7.8	19.4
18.6	7.2	5.8	22.1
18.6	9.4	5.7	28.5

* Each respiration trial was carried out for four days.

During most of the time that these studies were in progress, soybeans of high natural moisture content were not available; this made it necessary to resort to artificial conditioning in order to secure the desired range of moisture levels. Bailey (8), working on the respiration of cereal grains and flaxseed, carried out this procedure by determining the moisture content of the grain, adding the calculated amount of water, keeping samples at a temperature of 2° C., and mixing at frequent intervals during a period of at least three days while the moisture became evenly distributed throughout the sample. This procedure proved to be unsatisfactory with soybeans. It was difficult to secure uniform moisture distribution since some of the beans swelled very greatly, seed coats were loosened, and many beans were split. In order to obviate these difficulties, the sample was spread on a screen tray over water in a closed cabinet. The soybeans took up moisture from the saturated atmosphere and were removed from the cabinet when the weight of the sample indicated that the desired moisture content had been reached. The conditioned samples were kept in a cold room at a temperature of 2° C. for at least three days before determining their actual moisture contents and employing them for respiration studies.

An experiment was conducted to compare the effect on respiratory rate of conditioning with water vapor, as contrasted with the direct addition of water to the beans. It will be noted from the results given in table 5 that the direct addition of water gave higher rates in all cases and that the differential became greater with increasing moisture content. In the main experiments, where soybeans were artificially conditioned, the high-atmospheric-humidity method was used.

Table 5. Effect of Two Methods of Conditioning Soybeans on Respiration

Moisture content	Respiration at 37.8° C. CO_2/100 g. dry matter per 24 hours	
	Direct addition of water	Exposure to high humidity
per cent	mg.	mg.
11.8	1.3	1.1
13.5	2.4	2.1
15.2	6.1	4.0
17.6	32.6	10.2

Effect of Moisture Content on Respiration

In order to determine the effect of moisture content of soybeans on their rate of respiration, a number of samples from a lot of clean, sound 1939 Illini soybeans of 88 per cent germination

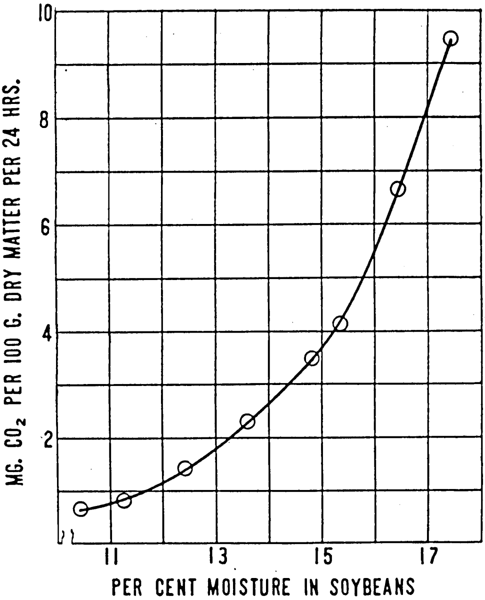

Fig. 3. Relation Between Rate of Respiration and Moisture Content of Soybeans Soon After Conditioning. (Respiration Data Are Based on Four Days Incubation at 37.8° C.)

were conditioned to various moisture contents, and their respiratory rates determined in the manner described above. Two or more replicates were used at each moisture level, and the smooth curve shown in figure 3 resulted from the means of these determinations. Table 6 was prepared by interpolating the respiratory rates at whole percentages of moisture.

It will be noted that there is no sharp break in this curve at any particular moisture value, such as found by Bailey and Gurjar (9) in the case of wheat respiration. These workers set the maximum moisture content for safe storage at the point where this break occurred. Obviously, further information is necessary to ascertain the maximum moisture content for the safe storage of soybeans, even when temperature is not a variable factor.

It must be emphasized that these data were obtained with sound beans under conditions which provided little opportunity for microorganisms to proliferate.

Table 6. Effect of Moisture Content on Respiratory Rate. Mean Respiration Rates for 1939 Illini Soybeans (88 per cent germination)

Moisture content	Respiratory rate at 37.8° C. CO_2/100 g. dry matter per 24 hours
per cent	mg.
10	0.6
11	0.8
12	1.1
13	1.7
14	2.5
15	3.7
16	5.4
17	8.0

Effect of Period of Dampness on Respiratory Activity

When several successive four-day respiration trials were carried out with the same samples, the respiratory rates at moisture contents below about 14 per cent remained virtually constant while those at the higher moisture levels showed a somewhat irregular but, neverthless, marked increase for each successive four-day period. This resulted in a progressively sharper inflection in the curve near the 14 per cent moisture level, those for the third and fourth periods being similar to typical respiration curves found by Bailey and Gurjar (9) for wheat and by Ramstad and Geddes (38) for oats. All samples showing increased respiration were sour or moldy upon removal from the respirometer.

In order to secure a better measure of the magnitude of such changes, 1939 Illini beans were conditioned to four moisture levels varying from 14.5 per cent to 16.2 per cent moisture and kept at a temperature of 37.8° C. for one month. At the end of that period, respiratory rates of the samples were determined and compared with those found soon after conditioning. The results given in table 7 show increases in respiratory rate of from 380 per cent to 740 per cent, depending on moisture content. All the samples showed evidence of mold growth.

The effect of temperature of storage on mold growth and the attendant increase in respiratory rate was demonstrated by conditioning samples from a lot of 1939 Illini soybeans to four different moisture contents and storing for three and one-half months at three different temperatures, 2° C., room temperature (approximately 25° C.), and 37.8° C. At the end of the storage period, respiratory rates of the various samples were determined at 37.8° C. These data are shown in table 8. The respiratory rates of the samples stored at 2° C. are practically the same as those found soon after wetting. These soybeans were free from visible mold, had good color, and a sweet odor. The samples stored at room temperature all showed some mold growth, though not more than a trace was present in the sample containing 13.8

Table 7. Effect of Time of Storage at 37.8° C. on Apparent Respiration of Soybeans

Moisture	Respiration at 37.8° C. CO_2/100 g. dry matter/24 hours	
	Stored 5 days	Stored 30 days
per cent	mg.	mg.
14.5	3.0	11.4
14.7	3.3	16.0
15.7	4.9	32.8
16.2	5.9	43.7

Table 8. Effect of Storing Soybeans for Three and One-Half Months at Different Moisture Contents and Temperatures on Respiration, Burton-Pitt Readings, and Germination Capacity

Number	Moisture	Temperature of storage	Respiration*	Burton-Pitt†	Germination
	per cent				per cent
IA	13.8	37.8° C.	10.6	101	0
IIA	14.9	37.8° C.	12.5	117	0
IIIA	15.8	37.8° C.	24.9	125	0
IVA	16.9	37.8° C.	72.8	142	0
IB	13.8	Room temp.	12.8	102	0
IIB	14.9	Room temp.	20.5	111	0
IIIB	15.8	Room temp.	25.5	122	0
IVB	16.9	Room temp.	44.2	133	0
IC	13.8	2° C.	2.8	101	70
IIC	14.9	2° C.	3.6	107	61
IIIC	15.8	2° C.	4.8	119	75
IVC	16.9	2° C.	7.8	126	75

* Respiration is expressed as mg. CO_2 produced per 100 g. dry matter per 24 hours at a temperature of 37.8° C.

† Burton-Pitt values are expressed on an arbitrary scale and hence are relative values applicable only to these data.

per cent moisture. The samples at 15.8 per cent and 16.9 per cent moisture were quite musty. All the samples stored at 37.8° C. were moldy, had a sour odor, and a brown color (which was very dark in the case of the 16.9 per cent moisture sample). Table 8 also includes germination data and the scale readings of these samples on the Burton-Pitt moisture tester.

This instrument is described by Burton and Pitt (15) and is a capacitance type moisture tester; the readings obtained depend upon the dielectric constant of the material being tested. Bailey (8) reported that the milliammeter readings obtained with the Burton-Pitt device are not perfectly correlated with moisture content of the grain being tested and appear to be in part a function of grain properties other than moisture content. He further stated that while respiratory rates vary to some extent independently of moisture, the same variables which affect respiratory level of grain also appear to influence the milliammeter readings in the same direction and to much the same degree, and thus such readings might be a more useful criteria of keeping qualities of grain in storage than any other single chemical or physical characteristic.

It is seen from table 8 that at equivalent moisture contents the samples exhibiting the greater respiratory activity gave somewhat higher Burton-Pitt readings, although this latter difference was by no means proportional to the difference in respiratory activity.

Table 9. Effect of Eleven Months' Storage at Room Temperature on the Respiration of Soybeans

Moisture content	Respiration at 37.8° C. CO_2/100 g. dry matter/24 hours	
	After eleven months' storage at room temperature	Four days after conditioning
per cent	mg.	mg.
9.0	0.9	......
10.7	1.3	0.7
11.3	1.4	0.9
11.7	2.4	1.0
12.3	4.6	1.3
15.0	17.4	3.7
15.8	31.6	5.0
17.1	66.5	8.2
19.8	172.0	......
20.9	280.0	......

Another experiment was carried out which gave a more complete picture of the effect of time of storage on the respiration of soybeans of various moisture contents stored at room temperature. Respiratory rates were determined with soybeans of various moisture contents which had been stored in airtight glass jars in the laboratory for 11 months. The results are shown in table 9 together with rates for freshly conditioned beans as interpolated from figure 3. Here, even the samples of low moisture content showed an increased respiratory rate.

It is of interest to ascertain whether storage of soybeans at high moisture contents would alter their respiratory activity when dried to lower moisture levels. A lot of 1939 Illini soybeans was conditioned to 16.4 per cent moisture and stored at room temperature for three months, then allowed to air-dry to various moisture contents, stored in airtight containers for four days in order that the moisture distribution might come to equilibrium, and the respiratory rates determined. The results are given in

Table 10. Respiratory Rates of Soybeans Stored for Three Months at Room Temperature at 16.4 Per Cent Moisture and Air-Dried to Various Moistures Compared with the Respiratory Rates of Soybeans at These Same Moisture Levels Shortly after Wetting

Moisture	Respiration at 37.8° C. CO_2/100 g. dry matter/24 hours	
	Dried samples	Original
per cent	mg.	mg.
10.9	1.6	0.8
12.9	2.3	1.6
14.1	8.7	2.7
14.4	11.0	3.0
14.9	14.0	3.6
15.1	19.7	3.9
16.4	39.4	6.4

table 10, together with the rates for freshly conditioned beans of the same moisture content, as interpolated from figure 3. It is seen that the respiratory rates of the air-dried soybeans were somewhat higher than those for freshly conditioned beans even at the lowest moisture content and diverged rapidly above 12.9 per cent moisture.

Comparison of Respiratory Activity of Naturally Damp and Artificially Dampened Soybeans

All the respiration studies described up to this point were conducted with soybeans which were artificially conditioned from moistures of 8 to 10 per cent up to the desired moisture levels. It seemed desirable to compare the respiratory activity of naturally and artificially dampened grain. In 1939 and 1940, weather conditions at harvest time in most soybean-growing areas were such that the beans had a low moisture content. In 1940, arrangements were made to obtain samples from Urbana, Illinois, which had not been allowed to dry in the field. The variety, moisture content, and respiratory rate of three samples received in this connection are given in table 11. The respiratory activity of these soybeans did not differ appreciably from that of beans which were artificially conditioned to the same moisture levels.

Table 11. Respiratory Rates of 1940 Crop Soybeans Soon after Harvest (natural moisture)

Variety	Moisture Content	Respiration at 37.8° C. CO_2/100 g. dry matter/24 hours
	per cent	mg.
Mandarin	12.3	1.5
Richland	12.6	1.6
Mukden	13.1	1.8

Climatic conditions in 1941 were markedly different, and samples of high moisture soybeans were readily obtained. The respiratory rates of some naturally damp beans are tabulated in table 12. Since these samples were obtained from commercial channels, nothing was known of their previous history. Their respiratory rates do not show a consistent relation to moisture content. It has already been shown that respiratory activity of soybeans is dependent not only on moisture content, but also on the temperature and length of the storage period. It would seem reasonable to attribute the apparently anomalous respiratory behavior of these samples to differences in their history, more particularly with the opportunity provided for the development of microorganisms.

Table 12. Respiratory Rates of Naturally Damp Soybeans (1941 Crop)

Moisture Content	Respiration at 37.8° C. CO_2/100 g. dry matter/24 hours
per cent	mg.
14.8	5.5
16.2	11.6
17.7	14.1
18.0	10.6
18.2	20.8
18.3	13.4
18.3	20.0
18.7	16.5
18.8	49.8
19.5	42.9
20.0	35.3

Influence of Temperature on Soybean Respiration

Temperature exerts a very marked effect on the respiratory activity of stored grain. Bailey and Gurjar (9) showed that the respiratory rate of wheat increased with temperature up to 55° C.; above 55° C. inactivation of enzymes proceeded very rapidly and caused a decrease in carbon dioxide production. The acceleration of respiration was not uniform but varied with the temperature. The most rapid change took place between 35° and 55° C. Coleman, Rothgeb, and Fellows (17) found that sorghum grains over a series of moisture levels respired about twice as rapidly at 37.8° C. as at 27.8° C.

An incubation temperature of 37.8° C. was employed in the soybean respiration experiments already described. It was recognized that such a temperature represents an extreme rather than a usual condition for stored grain. An extensive study of the effect of temperature on respiration was not undertaken, but a few observations showed how important temperature may be.

Soybeans were conditioned to 17.3 per cent moisture, and respiratory rates were determined at three different temperatures. Later, another sample from the same lot of beans was conditioned to 13.5 per cent moisture, and the respiratory rates were determined at five different temperatures. The results, which are shown in table 13, are too meagre to warrant drawing definite conclusions, but they seem to indicate that the accelerating effect of temperature on the respiration of soybeans is not uniform. This may be explained, at least in part, by variations in the number and kind of microorganisms present at different temperatures.

Table 13. Effect of Temperature on the Respiratory Rate of Soybeans

Moisture content	Temperature	Respiration CO_2/100 g. dry matter/24 hours
per cent	° C.	mg.
17.3	37.8	6.6
17.3	25	5.6
17.3	4	0.6
13.5	50	7.1
13.5	37.8	3.7
13.5	25	0.6
13.5	15	0.4
13.5	4	0.1

Respiration of Split Soybeans

In the U. S. standards for soybeans, the percentage of split beans is a grading factor. The maximum allowable amount of splits is less in the higher grades than in the lower; 30 per cent is allowed in grade No. 4, while the maximum in grade No. 1 is 10 per cent.

Bailey (7) showed that cracked corn had an appreciably higher respiratory rate than whole sound corn within the range of moisture contents studied (13.5 to 17 per cent). He expressed doubt that this increased rate of respiration was due to the mechanical injury in itself since corn in this moisture range has a much lower respiratory rate than those tissues in which a mechanical injury effect has been shown. Rather, he attributed the increase to improved conditions for gaseous diffusion to and from the respiring cells and to improved opportunity for growth of fungi on the surfaces of the broken fragments.

Very striking results were obtained in a study of the comparative respiratory rates of whole and split soybeans. A quantity of 1939 crop Illini soybeans was split by hand and conditioned to five moisture levels, varying from 13.9 per cent to 15.8 per cent. At the same time, whole soybeans from the same lot were also conditioned to approximately the same series of moisture contents. After storing for four weeks at room temperature (approximately 25° C.) respiratory rates were determined at 37.8° C. with the results shown in table 14. Within the range of moisture content covered by these experiments, the respiratory activities of the whole and split beans diverged rapidly. At 15.8 per cent moisture, the splits respired more than six times as rapidly as the whole beans of the same moisture stored under the same conditions and about twenty-four times as fast as whole soybeans of that moisture content four days after conditioning.

Table 14. Respiration of Split and Whole Illini Soybeans Four Weeks after Conditioning

Moisture content	Respiration at 37.8° C. CO_2/100 g. dry matter/24 hours	
	Whole beans	Split beans
per cent	mg.	mg.
13.9	3.0	6.3
14.3	7.2	15.2
15.1	....	73.0
15.7	....	98.2
15.8	....	120.4
15.9	17.9	....
16.3	31.1	....

Effect of Microorganisms on the Respiration of Soybeans

The soybean respiration experiments already described provide very strong circumstantial evidence that microorganisms are responsible for most of the respiratory activity observed in soybeans of high moisture content which have been kept at a relatively high temperature. In none of these experiments has the respiration of soybeans been separated from that of accompanying microorganisms.

Reference has already been made to the work of Larmour, Clayton, and Wrenshall (32), who attempted to evaluate the relative importance of these two factors in the respiration of stored grain by using treatments designed to kill or inhibit the growth of microorganisms. The difficulty in this method of attack lies in finding an agent or treatment which will have the desired effect on the microorganisms without altering the respiratory activity of the grain.

Experiments were conducted for the purpose of trying to sterilize soybeans without otherwise affecting them. Treatments involving washing the beans in aqueous solutions of disinfectants were unsatisfactory because the seed coats were cracked and the moisture content of the beans was changed. Toluene vapor was found to prevent mold growth, but it also caused loss of viability of the soybeans.

Calcium propionate is now finding wide commercial application as a mold inhibitor in dairy and bakery products. While it does not kill molds, this substance is a very efficient inhibitor of their growth for varying periods of time, depending on the concentrations used. An experiment was set up to determine the effect of dusting with calcium propionate on the respiratory activity of soybeans under conditions which would otherwise permit mold growth. Soybeans conditioned to 17.6 per cent moisture

Table 15. Effect of Dusting with Calcium Propionate on Soybean Respiration

Calcium propionate per 100 g. beans	Respiration at 37.8° C. CO_2/100 g. dry matter/24 hours
grams	mg.
0.0	59.8
0.1	68.2
0.2	61.8

were divided into three lots which were dusted with 0.0, 0.1, and 0.2 g. calcium propionate per 100 g. soybeans, respectively. The respiratory activities determined at 37.8° C., after allowing the samples to stand at room temperature for three weeks, are shown in table 15. The treatment with calcium propionate had no significant influence on respiratory rate, despite the fact that the control sample was very moldy while the dusted samples were free from visible mold. These results may possibly be due to the fact that calcium propionate is a good substrate for bacterial growth and the added stimulus to the development of bacteria offsets mold inhibition.

Soybean samples were submitted to the Division of Plant Pathology for examination. Surface sterilization techniques indicated that bacteria and fungi were present in the interior of the beans as well as on the surface. This is further evidence of the difficulties which must be overcome if such material is to be sterilized without injury.

Another line of attack was tried. Despite the fact that drastic heat treatment killing both soybeans and associated microorganisms would alter the characteristics of the soybeans as a substrate for the growth of microorganisms, it seemed worth-while to determine the respiratory rate of microorganisms on such a substrate. One hundred grams of beans was placed in each of two respirometers which were then heated for three hours in an air-oven at 130° C. After cooling, sufficient sterile water was added to one respirometer to bring the soybeans to an estimated moisture content of 19 per cent; to the other respirometer, the same quantity of inoculated water (prepared by shaking 25 g. of untreated moldy soybeans with 100 ml. water) was added. The respirometers were then incubated for nine days at 37.8° C. in order to allow microorganisms in the inoculated sample to proliferate. The atmospheres in the respirometers were replaced with carbon dioxide-free air, and the carbon dioxide produced in the next 24 hours was measured. The inoculated sample, which contained 19.0 per cent moisture, produced 68.5 mg. carbon dioxide whereas the "sterile" sample (19.8 per cent moisture) produced only 3.0 mg.

Since the "sterile" sample produced some carbon dioxide, the heat treatment did not destroy all biological activity, but the respiration was decreased to a very low level. On the other hand, the inoculated sample attained a rate of respiration that was in the range to be expected with untreated samples under similar conditions of moisture content, temperature, and time of storage.

EFFECT OF STORAGE CONDITIONS ON THE VIABILITY OF SOYBEANS

Considerable difficulty has been experienced by growers and seed companies in maintaining the viability of soybeans in storage.

Investigations of Simpson (41, 42) with cottonseed and of Robertson, Lute, and Gardner (39) with wheat, oats, and barley showed that the germination capacity of such grains decreased with increasing moisture content and with the time and temperature of storage.

Burlison, Van Doren, and Hackleman (14) investigated the effect of time and conditions of storage on the germination of soybeans. They found that the viability of soybeans stored in farm cribs depends not only upon the age of the seed, but upon the condition of the crib, the moisture content of the beans when stored, and the depth of the beans within the crib.

In order to study the effect of moisture content on loss of viability in soybeans, 1939 Illini soybeans were conditioned to 10 moisture levels varying from 9.4 per cent to 19.1 per cent. The samples were stored at room temperature in glass jars, and at intervals subsamples were submitted to the Minnesota State Seed Testing Laboratory for germination tests. For the early storage periods, the results of the successive germination trials were somewhat variable, and only data obtained at the end of three months' storage are recorded in table 16. It will be noted that the viability decreased markedly with increasing moisture content. After 20 months' storage all the samples were completely nonviable except the lowest moisture sample which germinated only 45 per cent.

Table 16. Germination of Soybeans after Three Months' Storage at Room Temperature (original germination 85 per cent)

Moisture content	Germination	Moisture content	Germination
per cent	per cent	per cent	per cent
9.4	78	14.6	4
10.5	67	15.5	1
11.8	37	16.6	0
12.6	41	18.0	0
13.7	9	19.1	0

Storage at low temperature may aid materially in maintaining the viability of soybeans at a high level. This is indicated by the studies summarized in table 8. When soybeans of moisture contents varying from 13.8 per cent to 16.9 per cent were stored for 3½ months at room temperature and at 37.8° C., none of the soybeans was viable. However, samples at the same moisture contents stored for the same length of time in a cold room at approximately 2° C. retained a high degree of viability. Tests, after 18 months' storage, showed the germination of these cold storage samples at moistures of 13.8, 14.9, 15.8, and 16.9 per cent to be 85, 84, 78, and 42 per cent, respectively.

In the experiments just described, the soybeans were kept in airtight jars. Under such conditions sufficient air may not have been available for normal respiration over a long period of time. However, even in the case of soybeans stored in sacks or metal cans with loosely fitting lids, a loss of 50 per cent in germination was observed after one year's storage at room temperature.

From the evidence, it appears that to maintain high germination in soybeans kept for seed purposes, they should be of low moisture content and stored at a low temperature.

EFFECT OF DAMAGE IN STORAGE ON THE CHEMICAL COMPOSITION OF SOYBEANS

Hall (25) has pointed out that in the evaluation of soybeans for commercial purposes the percentage of split and damaged beans plays an important part. In order to check the validity of the inspection methods used, he compared split, damaged, and whole sound beans by making certain chemical studies upon the crude oil and, to a lesser extent, upon the crude protein. As compared with whole soybeans, the splits contained slightly more crude oil and crude protein and the acid value of the oil was somewhat higher. Damaged beans, compared with sound beans, gave higher yields of crude protein and crude oil, and the oil was darker in color, materially higher in acid value, and lower in iodine value.

In order to determine the effect of moisture content on certain biochemical changes which take place in storage, Illini soybeans of the 1939 crop were conditioned to a series of moisture levels ranging from 9 per cent to 21 per cent and stored in glass jars at room temperature for 12 months. At the end of the storage period, the beans were air-dried, ground in a Wiley laboratory mill to pass the 1 mm. sieve, and submitted to determinations of *p*H, reducing and nonreducing sugars, oil content, oil iodine value, nonprotein nitrogen, and catalase activity.

The *p*H of the samples was determined by shaking the ground meal with 10 times its weight of water, allowing it to stand for half an hour, filtering, and determining the *p*H of the filtrate with a glass electrode. Reducing and nonreducing sugars were determined by the ferricyanide reduction method as described by the American Association of Cereal Chemists (1). This method was designed for flour analysis—and the reducing and nonreducing sugars are expressed as maltose and sucrose, respectively; stating the results in terms of these two sugars should not be construed as indicating the nature of the sugars present in soybeans. Oil content was determined by Butt tube extraction with petroleum ether (Skellysolve F) as the solvent; 5-gram samples were extracted for two hours, reground with sand, and the extraction continued overnight. Iodine numbers were calculated from the refractive indices determined with a Zeiss dipping type refractometer using the equation of Majors and Milner (33) where: Refractive Index (nD^{25}) $= 1.45755 + 0.0001179$ Iodine Value. Nonprotein nitrogen was determined by the method of Becker, Milner, and Nagel (12). Catalase activity was measured by the volume of oxygen released from 5 ml. neutralized hydrogen peroxide in 15 minutes at 25° C. by 0.5 g. meal (dry matter basis).

The results of these analyses are given in table 17. In considering these data it should be mentioned that the samples which had been stored at 15 per cent and higher moistures showed visible

Table 17. Biochemical Properties of Soybeans after Storage at Room Temperature for One Year at Various Moisture Contents

Moisture content during storage	pH	Reducing sugar*	Non-reducing sugar†	Oil‡	Iodine number	Non-protein nitrogen§	Catalase activity‖
per cent		mg.	mg.	per cent		mg.	ml.
9.0	6.62	99	1048	22.1	129.6	2.6	30.7
10.7	6.50	89	1045	22.3	127.6	2.6	21.2
11.7	6.58	89	1035	22.3	129.4	2.6	9.5
12.3	6.56	90	1042	22.7	127.5	2.7	3.4
15.0	5.82	101	942	22.6	127.5	2.7	2.4
15.8	6.34	145	855	22.6	125.9	3.2	5.4
17.1	6.19	262	620	22.7	104.9	2.9	8.0
19.8	6.32	289	335	23.7	89.1	2.6	12.0
20.9	6.62	225	312	24.0	100.9	2.7	11.6

* Reducing sugar expressed as mg. maltose per 10 g. dry matter.

† Nonreducing sugar expressed as mg. sucrose per 10 g. dry matter.

‡ Oil contents expressed on dry-matter basis.

§ Nonprotein nitrogen expressed as mg. N per g. dry matter.

‖ Catalase activity expressed as ml. O_2 produced from 5 ml. neutralized 4 per cent H_2O_2 in 15 minutes at 25° C. by 0.5 g. (dry matter basis) ground meal.

mold growth; the three highest moisture samples were completely covered with molds. Of the various biochemical properties studied, changes in the carbohydrates seem to be the most closely related to increased respiratory activity. The amount of reducing sugars was more than doubled in going from the lowest to the highest moisture content, while the concentration of nonreducing sugars decreased to less than one third that found in the lowest moisture sample. It would appear that the oil content remained essentially constant in all samples. The apparent increase in oil content in the high moisture samples may be accounted for on the basis of the decrease in total carbohydrate, so that while the absolute amount of oil remained constant, its proportion relative to the total weight increased slightly. Iodine number of the oil decreased with increasing moisture, indicating that storage of soybeans at high moisture contents may have an adverse effect on the drying value of the oil. Nonprotein nitrogen did not vary significantly with differences in moisture content.

The results of the catalase activity determinations are of interest. Catalase activity markedly decreased with increasing moisture content to a minimum value in the sample stored at 15.0 per cent moisture; further increases in moisture resulted in increased catalase activity. The initial decrease is correlated with the decrease in viability with increasing moisture observed in previous experiments. On the other hand, the subsequent increase is correlated with increasing numbers of microorganisms observed in the samples stored at moistures above 15 per cent.

From these data it would appear that carbohydrates are the chief materials metabolized by stored soybeans. At high moisture contents most of the nonreducing sugars have apparently been converted to reducing sugars which, in turn, are used in respiration, but at a somewhat less rapid rate than they are produced, which results in an increase in reducing-sugar concentration.

ADIABATIC STORAGE STUDIES

When high-moisture grain is stored in bulk, heating may take place which would not occur in a small sample of the same lot of grain. This is because of the so-called "mass effect"; grain is a relatively poor heat conductor and since loss of heat is proportional to the surface, the loss is reduced when the mass is increased. Heating of grain may, however, be studied even when the sample is small if some means is provided to prevent loss of heat.

Several workers have measured heat production in various organic materials by using Dewar flasks to reduce the rate of heat loss. James (28) devised an apparatus which consisted of a Dewar flask to contain the material being studied and a means for slowly aerating the material. Using corn meal of high moisture content, little heating was observed unless the sample was aerated. Samples which heated were always moldy at the conclusion of the experiment. Investigations of James, Rettger, and Thom (29) employing this technique demonstrated the importance of microorganisms in the heating phenomenon.

Gilman and Barron (23) noted that bin-burned grain was invariably moldy. Their experimental observations indicated that marked increases in the temperature of stored grain may be ascribed to mold growth. Quite large variations were noted in the thermogenic powers of different molds growing on the same grain and also in the heat production by a particular mold growing on different grains.

Bakke and Noecker (10) studied the relation of moisture to respiration and heating in stored oats. Respiratory rates were determined by measuring oxygen uptake while heating was followed by measuring the temperature increase of oats kept in stoppered Dewar flasks immersed in a water bath maintained at 25° C. No means was provided for aerating the flasks. While, in general, respiratory rate and heating were found to increase with increasing moisture content, a great deal of variation was observed between samples of the same moisture content. The authors attributed these apparent discrepancies to variations in the mold populations of the various samples. Many workers have shown that aeration is essential to heating and it is therefore surprising that Bakke and Noecker secured any appreciable heating since their grain was stored in stoppered flasks. They apparently assumed that aeration does not take place in bulk storage; evidence will be presented later which indicates that, even in very large elevator bins, grain is probably slowly aerated.

Trials in this laboratory demonstrated that the heating of grains in aerated Dewar flasks kept at constant temperature could be observed only if the moisture content was considerably in excess of moisture levels at which grain is known to heat in commercial storage. In investigating the heating of soybeans, it appeared desirable to construct apparatus which would simulate as closely as possible conditions found in the interior of a large mass of grain. With this object in view, a large scale adiabatic respirometer was constructed and employed for heating studies. Limitations of this device led later to the development of a small-scale adiabatic respirometer in which the experimental conditions could be more adequately controlled.

Large Adiabatic Respirometer Experiments

The large adiabatic respirometer is shown in figure 4. It consisted of two concentric insulated cylinders, the inner or grain chamber having a capacity of approximately six bushels. An insulated cover fitted tightly over the top of both cylinders, and an outside duct containing a fan and electric heaters provided a means for circulating and heating the air in the space between the two cylinders.

The automatic temperature control device consisted of two 200 ml. glass bulbs joined by a U-shaped length of capillary tubing into which two platinum contacts were sealed. The capillary tube contained sufficient mercury to complete a circuit between the platinum contacts. One bulb of the apparatus was in the grain chamber and the other in the air space between the inner and outer cylinders. When the device was in operation, a very slight increase in the temperature of the grain expanded the air in the inner bulb which forced the mercury away from one of the contacts in the capillary tube and operated a relay which closed the heater circuit. When the air in the outer chamber attained the temperature of the grain, expansion of air in the outer bulb actuated the

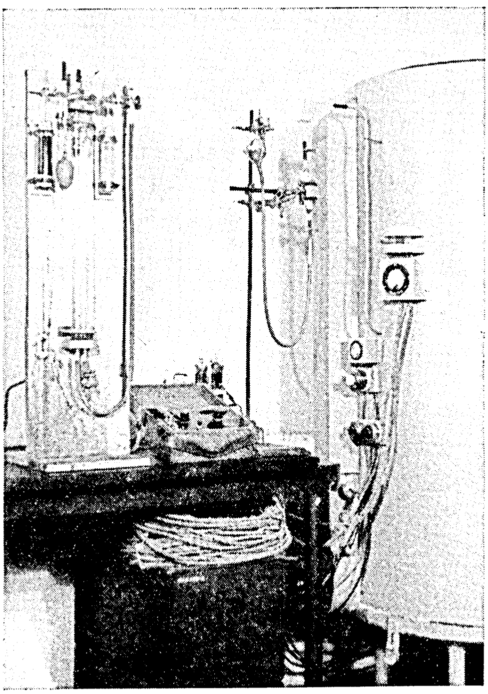

FIG. 4. LARGE ADIABATIC RESPIROMETER AND HALDANE-HENDERSON GAS ANALYSIS APPARATUS

control mechanism and interrupted the heater circuit. Actually the conditions maintained were not quite adiabatic. A certain amount of lag in operation permitted the grain chamber to become very slightly warmer than the outer chamber, but the apparatus was so adjusted that the reverse situation never occurred. Thus any danger of heating the grain by the surrounding air was eliminated. When high temperatures were being maintained, continuous heaters in the air duct supplied most of the necessary heat, and only one or two heaters were controlled by the relay; this lessened the lag in the operation of the control device.

The temperature differential between the two chambers was checked by means of a microammeter to which was connected a copper-constantan thermopile with 20 junctions in each chamber. In addition to this, four resistance thermometers spaced one foot apart in the center of the inner chamber were used to measure grain temperature. Further check on temperature control was provided by two resistance thermometers in the air chamber.

The sample received adequate aeration because the grain chamber was not airtight, and air circulating rapidly around it speeded up diffusion into and out of the chamber. This was demonstrated by withdrawing samples of air from within the grain and analyzing them for carbon dioxide and oxygen in a Haldane-Henderson apparatus. The carbon dioxide concentration never rose above 6 per cent, and the oxygen concentration was always above 14 per cent.

Soybeans were artificially conditioned to the desired moisture levels by spreading them in a layer about three inches deep on the floor of an experimental millroom equipped with a Bahnson humidifier, set to maintain a relative humidity of 90 per cent. Conditioning of the beans was accelerated by spraying with water at intervals and mixing by frequent shoveling. When the desired amount of water had been added, the soybeans were placed in large metal cans and the partially filled cans rolled in order to secure thorough mixing. In filling the respirometer, soybeans were removed from each of these cans in rotation.

Employing a commercial lot of soybeans, four heating trials were carried out at moisture levels of 14.7, 15.6, 17.5, and 18.8 per cent, respectively. The beans at 14.7 per cent moisture content failed to heat in 64 days even though a period of warm weather shortly after the trial was begun raised the temperature of the beans to 32° C. These beans were sound and free from mold upon removal from the respirometer and were employed for the trial at 15.6 per cent moisture.

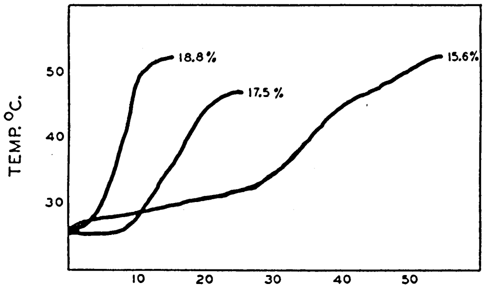

FIG. 5. EFFECT OF MOISTURE CONTENT ON THE RATE OF HEATING OF SOYBEANS IN THE LARGE ADIABATIC RESPIROMETER

The results of the heating trials are shown graphically in figure 5. The beans stored at 15.6 per cent moisture increased from an initial temperature of 27° C. to a maximum of 52° C. in 53 days; those at 17.5 per cent moisture rose from 26° C. to 47° C. in 23 days when the trial was terminated. The beans stored at 18.8 per cent moisture heated more rapidly and rose from a temperature of 26° C. to 52° C. in 14 days at which time the respirometer was emptied. From these results it would appear that soybeans having a moisture content of over 15 per cent, as determined by the two-stage vacuum-oven method, are liable to heat in storage.

Small Adiabatic Respirometer Experiments

The large adiabatic respirometer possessed a number of disadvantages and limitations. It required large quantities of soybeans and, aside from the cost and labor involved, it was difficult to condition them uniformly. Aeration of the sample was uncontrolled, rendering it impossible to measure respiration. These disadvantages were overcome by the construction of a small adiabatic respirometer patterned after equipment designed and employed by Dr. Earl B. Working at Kansas State College.[4] Photographs of this apparatus are shown in figures 6, 7, and 8.

The device operated on the same principle as the large respirometer previously described, in that heat loss was minimized by keeping the air surrounding the grain container at very nearly the same temperature as the grain by means of a very sensitive temperature control mechanism. A one quart commercial-type Dewar flask, employed as the respirometer, was placed in a well-insulated thermostat equipped with electric light bulbs, which served as heaters, and a circulating fan.

The air thermostat was constructed of ¾-inch plywood and insulated with two ½-inch layers of insulating board. Its inside dimensions were 14 inches high, 14 inches deep, and 18 inches wide. The heaters were separated from the main compartment

[4] Private communication.

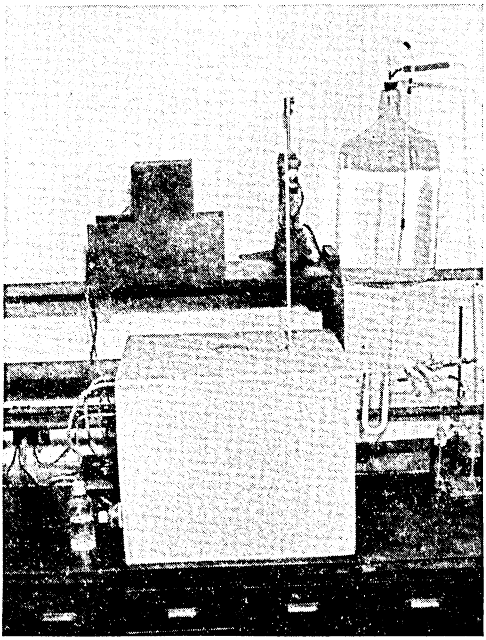

FIG. 6. SMALL ADIABATIC RESPIROMETER

by a partition containing two openings, the lower of which was fitted with an externally driven 4-inch fan. Provision for heating consisted of four electric light bulbs. Three of these were controlled by a three-heat switch mounted outside the thermostat and were used to supply most of the heat when the temperature was appreciably above room temperature. The fourth light bulb was connected with the temperature control circuit. Tap water was passed through a copper coil to prevent the temperature in the thermostat from rising, as a result of the motion of the fan or an increase in room temperature, before the sample started to heat.

Maintenance of adiabatic conditions was secured by means of a 24 junction copper-constantan thermopile connected to a sensitive D'Arsonval galvanometer (G. M. Laboratories Model No. 2551 B, provided with an integral light source), the light beam of which actuated a photoelectric cell which in turn operated a relay controlling the heater. Twelve of the thermopile junctions were centrally placed within the flask and the remainder disposed against the outer wall of the flask. When the soybeans became very slightly warmer than the air surrounding the Dewar flask, a current was caused to flow through the thermopile deflecting the galvanometer mirror and causing a beam of light to fall on the photoelectric cell, thus activating the relay controlling the heater. A stop was installed in the galvanometer limiting the rotation of the coil so that the light beam could not overshoot the photoelectric cell. As the temperature of the grain and the air in the thermostat became equalized, the light beam receded from the window of the photoelectric cell and the heater circuit was interrupted. A detailed description of the photoelectric relay and a diagram of the circuit has recently been given by Working (45).

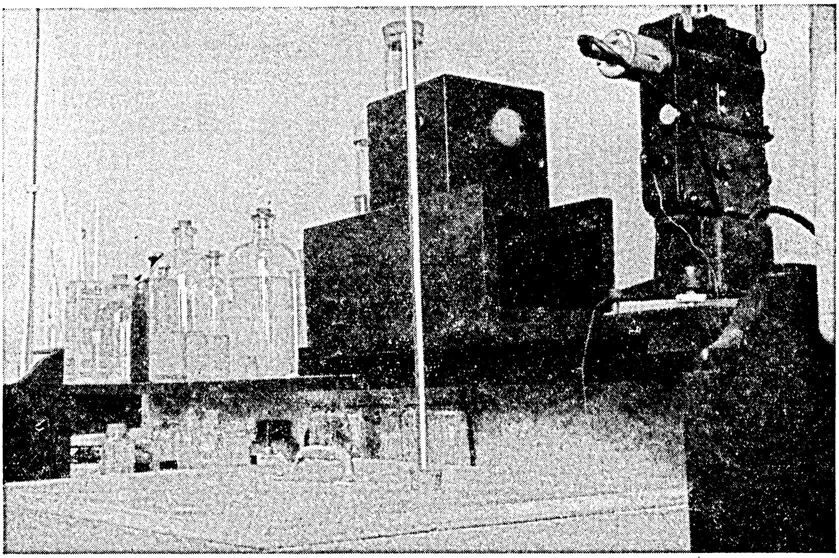

FIG. 7. GALVANOMETER WITH INTEGRAL LIGHT SOURCE AND PHOTOELECTRIC RELAY FOR TEMPERATURE CONTROL OF SMALL ADIABATIC RESPIROMETER

As shown in figure 7 the photoelectric cell, relay, and accessory equipment were assembled in a metal box with a window to admit light to the photoelectric cell. This box fitted snugly into wooden guides which were securely fastened to the shelf upon which the galvanometer was placed. The distance between the galvanometer mirror and the window was 10 inches.

Aeration of the soybeans was accomplished by an air inlet near the top of the Dewar flask and an outlet tube extending nearly to the bottom. Water was permitted to siphon very slowly from a five-gallon bottle drawing air through a strong alkali solution (which removed carbon dioxide from incoming air), two bottles of sulfuric acid-water solution (which humidified the air), the sample flask, a U-tube containing magnesium perchlorate (which removed moisture from the air), another U-tube containing ascarite (sodium hydroxide on asbestos fibers which removed the carbon dioxide given off by the sample) and magnesium perchlorate, and a solution of barium hydroxide (which served as a check on the absorption efficiency of the ascarite). The rate of aeration varied from 0.5 to 3.0 liters per 24 hours, depending upon the respiratory activity of the sample.

Before starting an experiment, the temperature control apparatus was carefully adjusted by filling the Dewar flask with water at 50° to 55° C. and setting the zero point of the galvanometer so

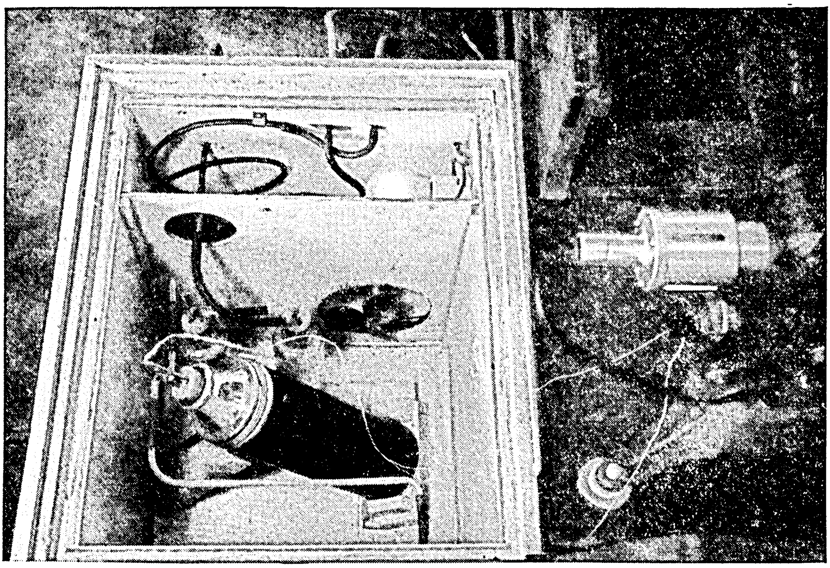

FIG. 8. INTERIOR VIEW OF AIR THERMOSTAT SHOWING DEWAR FLASK, THERMOPILE, HUMIDIFYING VESSELS, HEATER COMPARTMENT, AND FAN

that the system showed a temperature decrease of only 0.1° to 0.2° C. per 24 hours. While this meant that strictly adiabatic conditions were not maintained, it eliminated the possibility of the grain being heated by the surrounding air.

Preliminary experiments were conducted with two samples of soybeans. One of these had been stored for several months at 19.3 per cent moisture and was very moldy; surprisingly, it failed to heat when placed in the adiabatic respirometer for eight days. Apparently the flora present were not thermogenic. James, Rettger, and Thom (29) have reported finding organisms which proliferated freely but did not produce heating even though other strains of the same organisms were powerfully thermogenic. The other preliminary trial was carried out with a 500 g. sample of sound 1939 Illini soybeans conditioned to a moisture content of 19.9 per cent and placed in the respirometer immediately. After eight days the beans started to heat and reached a temperature of 44.5° C. on the eighteenth day when the experiment was ended.

An unseasonable blizzard on November 11, 1940, caused soybeans in some sections to be left in the field until the following February and March. When harvested, these beans contained an excessive amount of moisture and they were quite severely weather damaged. A sample of such soybeans containing 24.5 per cent moisture was obtained. It was somewhat moldy when

Table 18. Results of Heating Experiments with 600 g. Samples of Naturally Damp Soybeans (moisture content 24.5 per cent) in Small Adiabatic Respirometer

Day	Experiment No. 1		Experiment No. 2		Experiment No. 3	
	Temperature	Respiration*	Temperature	Respiration*	Temperature	Respiration*
	° C.		° C.		° C.	
Start	22.6	....	28.7	....	19.1	....
1	23.7	....	29.5	46.8	20.5	44.0
2	25.0	....	30.7	83.7	22.6	63.0
3	27.0	73.0	29.5	66.9	25.1	81.8
4	29.8	48.8	32.0	59.6	27.5	56.1
5	32.4	78.4	34.9	....	30.1	92.8
6	35.5	97.4	36.5	53.8	31.6	149.1
7	37.0	103.8	40.7	110	36.0	164.0
8	39.9	71.1	44.6	130.3	39.8	139.0
9	43.7	147.0	46.4	65.8	43.8	136.8
10	46.0	117.8	46.7	30.5	45.5	91.3
11	48.8	128.7	46.3	....	47.0	97.8
12	50.9	81.1	46.8	54.3	49.8	75.8
13	51.9	84.8	47.2	65.4	52.0	58.3
14	55.0	....	48.3	18.1	53.8	35.8
15	55.2	....	48.7	50.1	56.3	30.9
16	55.5	....	49.9	46.8	58.9	38.6
17	58.7	....	50.2	85.0	62.4	42.4
18	62.3	....	53.9	145.0	66.1	67.1
19	67.3	....	56.8	101.0	68.0	56.9
20	72.2	....	58.6	72.5	70.9	36.3
21	75.5	....	59.9	70.2	74.6	120.0
22	79.5	....	60.6	62.6	77.7	75.1
23	80.5	....	61.9	48.6	79.7	86.8
24	80.7	....	62.8	78.8	83.5	138.6
25	80.8	....	64.2	65.4	89.0	105.1
26	81.3	....	64.8	76.4	86.0	137.1
27	....	....	65.2	64.5	88.5	143.3
28	....	....	65.8	81.8	....	....
29	....	....	66.1	85.7	....	....
30	....	....	66.1	72.0	....	....
31	....	....	65.7	85.6	....	....
32	....	....	65.6	68.9	....	....
33	....	....	65.1	70.2	....	....
34	....	....	64.8	70.2	....	....

* Expressed as mg. carbon dioxide respired per 100 g. dry matter per 24 hours.

received and was stored at approximately 2° C. until required. Temperature and respiration data from three experiments with 600 g. samples of these beans in the small adiabatic respirometer are shown in table 18. Heating began immediately in all the experiments. The maximum temperatures of 81.3, 64.8, and 88.5° C. were much higher than those observed previously in the large adiabatic respirometer.

A recheck was made on the operation of the temperature control mechanism at the conclusion of each of the experiments, and it was found to be functioning properly, indicating that the high temperatures observed were not caused by the samples being heated from without.. Probably the most significant fact to be observed in table 18 is that respiratory activity did not show a steady increase with temperature as might have been expected if respiration of the soybeans alone had been involved. Wide fluctuations in respiratory rate from day to day show no direct relationship to temperature. On the third and eleventh days of the second experiment the aeration was discontinued, and the drop in temperature which occurred on both occasions shows that an adequate supply of air is essential to the heating process. For some unknown reason this sample failed to rise in temperature after the thirtieth day, although it continued to produce carbon dioxide at a relatively high rate. In this connection it is of interest to recall that James, Rettger, and Thom (29) found that the thermogenic powers of various strains of microorganisms growing on corn differed widely and that certain strains lost their ability to produce heat when sub-cultured for several weeks.

Table 19 gives temperature and respiration data on a 550 g. sample from this same lot of soybeans which was partially air-dried to a moisture content of 19.7 per cent. This sample heated more slowly than any of the samples at the original moisture content of 24.5 per cent, and the average respiratory rate was lower.

When a 400 g. sample of split soybeans which had been kept at room temperature in an airtight container at a moisture content of 16.2 per cent for seven months was placed in the small adiabatic respirometer, no heating occurred. The experiment was begun at a temperature of 23.6° C. During the first two days the average rate of carbon dioxide production was 9.1 mg. per 100 g.

Table 19. Results of Heating Experiment with 550 g. Sample of Naturally Damp Soybeans Partially Air-Dried from 24.5 Per Cent to 19.7 Per Cent Moisture

Day	Temperature	Respiration*	Day	Temperature	Respiration*
	° C.			° C.	
Start	23.4	....	16	44.0	56.6
1	23.8	9.9	17	45.5	85.0
2	24.6	30.4	18	46.4	35.3
3	25.6	30.4	19	46.8	39.8
4	26.8	47.1	20	47.3	19.7
5	28.2	30.6	21	50.3	33.5
6	29.7	54.6	22	50.7	15.2
7	31.3	43.4	23	51.0	8.6
8	33.1	60.7	24	51.6	22.6
9	34.1	44.6	25	52.1	9.5
10	35.7	53.2	26	52.4	23.3
11	37.3	22.6	27	53.5	16.0
12	38.9	86.0	28	53.8	14.5
13	39.8	24.7	29	54.3	18.5
14	41.0	42.5	30	55.1	18.6
15	42.6	55.7			

* Expressed as mg. carbon dioxide respired per 100 g. dry matter per 24 hours.

dry matter per 24 hours. In the next three days this rate dropped successively to 3.0, 1.8, and 0.45 mg. After four days at the latter rate of respiration the experiment was discontinued.

The result of this experiment was quite unexpected in view of information which had been obtained on the respiration of split soybeans at 37.8° C. The respiratory rate of a sample from the same lot of splits was then determined at 37.8° C. During the first four days the rate was 9.1 mg. carbon dioxide per 100 g. dry matter per 24 hours, and in the next four days the carbon dioxide produced exceeded the capacity of the absorbers and could not be measured accurately, but the respiratory rate was over four times as great as during the first four-day period. This indicates that at 37.8° C. very rapid growth of microorganic flora took place which did not occur at 23.6° C. in the adiabatic respirometer.

A 500 g. sample of soybeans was placed in the small adiabatic respirometer 24 hours after conditioning to a moisture content of 16.7 per cent. Temperature and respiration data from this experiment are presented in table 20. After the eighteenth day an ap-

Table 20. Results of Heating Experiment with 500 g. Sample of Soybeans Artificially Conditioned to 16.7 Per Cent Moisture

Day	Temperature	Respiration*	Day	Temperature	Respiration*
	° C.			° C.	
Start	24.2	....	26	24.9	6.7
1	24.1	....	27	24.9	6.5
2	24.5†	1.9	28	25.0	6.7
3	24.3	8.2	29	25.1	17.3
4	24.1	6.5	30	25.2	6.5
5	23.8	4.8	31	25.3	....
6	23.6	1.7	32	25.3	7.9
7	23.5	1.9	33	25.3	5.3
8	23.3	1.2	34	25.4	12.0
9	........‡	1.4	35	25.4	9.9
10	....	1.4	36	25.4	7.4
11	....	2.8	37	25.4	6.7
12	....	1.7	38	25.5	9.4
13	....	1.7	39	25.5	....
14	24.7	3.8	40	25.6	....
15	24.6	2.4	41	25.6	....
16	24.5	2.4	42	25.7	....
17	24.5	2.4	43	25.9	....
18	24.5	8.9	44	25.9	13.9
19	24.5	3.4	45	25.9	5.8
20	24.5	6.2	46	37.6§	7.9
21	24.5	6.0	47	37.9	15.4
22	24.6	7.0	48	38.2	18.0
23	24.6	7.4	49	38.6	8.9
24	24.7	7.0	50	38.9	19.2
25	24.8	5.0	51	39.0	23.8

* Expressed as mg. CO_2/100 g. dry matter/24 hours.
† Cooling water off caused this temperature rise.
‡ Temperature control mechanism not functioning from 9th to 13th day.
§ Heaters turned on to produce this temperature rise.

preciable increase in respiratory rate occurred but the temperature rose slowly. Between the forty-fifth and forty-sixth days the continuous heaters in the thermostat were turned on and the resulting 12° C. increase in temperature caused a marked acceleration in the rates of heating and respiration.

BULK STORAGE OF SOYBEANS

Large quantities of high moisture soybeans appeared on the market in the fall of 1941 as a result of damp weather at harvest time. This fact, together with the cooperation of a local soybean processor, made it possible to check the laboratory data on respiration and heating against the behavior of soybeans in bulk storage. For experimental purposes an interstitial bin was filled to a depth of 97 feet with 987,000 pounds of soybeans with an average moisture content of 15.82 per cent (Tag-Heppenstall). The lowest and highest moisture lots in the bin were 14.0 and 18.0 per cent moisture as determined by the Tag-Heppenstall electric moisture meter.[5] Table 21 shows the weights, grades, and

Table 21. Description of Soybeans in Elevator Bin

Weight of carload	Grade	Moisture*	Approximate depth in bin
pounds		per cent	feet
57,680	4	18.0	surface- 5
116,350	4	17.3	5 -17.5
64,500	3	14.5	17.5-23.5
78,580	3	15.3	23.5-31
60,000	3	15.9	31 -37
79,480	4	17.8	37 -45
82,970	3	15.9	45 -53
82,200	3	14.3	53 -61
80,900	3	15.2	61 -69
59,800	2	14.0	69 -75
123,440	4	16.0	75 -87
100,940	3	15.0	87 -97

* Determined with Tag-Heppenstall moisture meter.

moisture contents and relative depths of the various lots in the bin. Of these soybeans, 6 per cent graded U. S. No. 2, 56 per cent graded U. S. No. 3, and 38 per cent graded U. S. No. 4.

The filling of this bin was begun November 14 and completed November 19, 1941. At intervals during the storage period, a ½-inch steel pipe with a perforated tip was forced into the beans for the purpose of withdrawing gas samples and reading tempera-

[5] For a comparison of moistures as determined by the Tag-Heppenstall method and other methods see table 1, page 9.

Table 22. Temperatures Observed at Various Depths below Surface of Soybeans in Elevator Bin

Depth below surface	Date sampled and number of days after storage						
	Nov. 26	Dec. 2	Dec. 16	Dec. 26	Jan. 5	Jan. 13	Jan. 20
	7 days	13 days	27 days	37 days	47 days	55 days	62 days
Feet	° F.	° F.	° F.	° F.	° F.	° F.	° F.
5	45	45	40	41	34	45	52
10	46	46	45	47	55	59	55
15	46	47	45	48	54	61	58
20	47	47	46	48	56	65	60
25	48	47	47	49	57	67	62
30	48	47	47	50	58	69	64
35	48	47	48	50	60	69	78
40	46	47	47	50	62	67	99
45	48	48	47	50	60	64	101
50	48	49	47	50	58	78	82

tures at the various levels. No readings could be taken more than 50 feet below the surface since this was the maximum depth to which four men employing special clamps were able to force the pipe. Temperatures were taken by lowering a thermocouple into the pipe. Gas samples, obtained by connecting a sample tube and vacuum pump to the upper end of the pipe, were analyzed for carbon dioxide and oxygen with a Haldane-Henderson apparatus.

Table 22 shows the temperatures recorded to the nearest degree Fahrenheit at various depths below the surface. Analyses of gas samples drawn from various depths are given in table 23. During the first month's storage, the temperature remained practically unchanged, and very little carbon dioxide was found in gas samples removed from various depths down to 50 feet below the surface. During the second month, however, the temperature increased rapidly, and a temperature of 99° F. was attained 40 feet below the surface; at this level the greatest change in gas composition had taken place: 16.9 per cent carbon dioxide and 1.0 per cent oxygen. It should be noted that, in this study, the soybeans were cool when placed in storage, and the outside temperatures were always low. During the first week in January, the outside temperatures ranged from 0° to —20° F. and were responsible for the low temperature reading at the five foot depth on January 5. More than five feet below the surface the soybean temperatures did not appear to be greatly affected by the outside temperature.

The oxygen and carbon dioxide contents of the interseed atmosphere at various depths indicated that ventilation was taking place throughout the bin. The relative changes in concentrations of oxygen and carbon dioxide were considerably less than would

Table 23. Composition of Interseed Air at Various Depths below Surface of Soybeans in Elevator Bin

		Date sampled and no. of days after storage				Date sampled and number of days after storage			
Depth	Gas	Dec. 2	Dec. 9	Depth	Gas	Dec. 26	Jan. 6	Jan. 13	Jan. 20
		13 days	20 days			37 days	48 days	55 days	62 days
Feet		Per cent	Per cent	Feet		Per cent	Per cent	Per cent	Per cent
2.5	CO_2	0.31	0.27	5	CO_2	0.55	0.51	0.80	1.90
2.5	O_2	19.99	20.10	5	O_2	19.71	19.69	19.73	18.07
8	CO_2	0.40	0.35	10	CO_2	0.80	8.56	1.01	2.01
8	O_2	19.80	20.10	10	O_2	19.18	17.01	19.39	17.60
16	CO_2	0.46	0.31	20	CO_2	0.91	3.13	4.34	4.29
16	O_2	19.57	20.10	20	O_2	18.66	16.51	14.90	14.84
24	CO_2	0.57	0.33	30	CO_2	1.54	4.17	5.37	6.03
24	O_2	19.48	20.10	30	O_2	17.76	15.89	13.46	11.94
32	CO_2	....	0.38	40	CO_2	3.14	2.09	10.61	16.93
32	O_2	....	20.10	40	O_2	16.16	18.13	8.16	1.05
				50	CO_2	1.39	1.02	3.58	8.40
				50	O_2	18.06	19.29	14.73	8.82

be expected from laboratory studies of the respiratory activity of soybeans undergoing heating.

On January 18 a sour odor emanating from the bin gave evidence that spoilage might be greater than the temperature would indicate. On January 20, 62 days after the experiment was begun, the bin was emptied and composite samples taken at several time intervals. Soybeans from the bottom of the bin were cool and sweet although the moisture content determined by the two-stage vacuum-oven method was 17.8 per cent (Tag-Heppenstall value 16.0 per cent). Another sample, representing a later period during the emptying of the bin, which likewise appeared to be undamaged, had a moisture content of 19.2 per cent (Tag Heppenstall value 17.2 per cent). These samples rapidly became moldy when stored at room temperature. Although the highest temperature observed was 101° F. (38.3° C.), about 40,000 pounds of beans were damaged as evidenced by a brown discoloration and a sour odor. A sample of these soybeans had a moisture content of 20.3 per cent, while a small quantity of very severely damaged beans contained 28.0 per cent moisture, as determined by the two-stage vacuum-oven method; the moisture contents of these samples were outside the range of the Tag-Heppenstall moisture meter. From the moisture values of the four samples it would appear that the beans were not adequately sampled before storage; the difficulty of obtaining a representative sample from a carload of damp soybeans by the usual probing methods is well recognized by the trade.

The two samples of damaged soybeans were submitted to the Division of Plant Pathology to determine the nature of the micro-organisms present. Bacteria and fungi were both found in abundance. The bacteria were not identified but were considered to be the agents responsible for the brown discoloration of the damaged soybeans and for the sour odor which was present. Surface sterilization techniques indicated that the bacteria and fungi were present not only on the surface of the beans but in the interior as well. The following fungi were identified as being present in these samples:

Alternaria sp.
Fusarium sp.
Penicillium sp.
Aspergillus repens group
Aspergillus niger group
Chaetotheca sp.
Verticillium sp.
Aspergillus flavus-oryzae group
Mucorale
Rhizopus nigricans
Acrostalagmus sp.

From laboratory studies of respiratory rate, it seems evident that if the soybeans had been stored at a higher initial temperature, or for a longer time, very extensive damage would have occurred. The fact that there was some spoilage with a maximum recorded temperature of 101° F. indicates that soybeans may undergo considerable damage without excessive heating.

This experiment lends further proof to the conclusion, earlier drawn from laboratory experiments, that there is a definite risk involved in storing soybeans at moisture contents permitted in the grade definitions for Nos. 3 and 4, namely 16 and 18 per cent, as determined by the Tag-Heppenstall moisture meter.

PRODUCTION OF CARBON MONOXIDE BY HEATING SOYBEANS

Very little is known regarding the nature of the volatile substances, other than carbon dioxide, produced in a mass of heating grain or of the processes by which they are formed.

As far as the authors are aware, no authenticated reports of the production of carbon monoxide in heating grain are in the literature. Langdon (30) and Langdon and Gailey (31) found from 1 to 12 per cent of this gas in the hollow pneumatocysts of *Nereocystis luetkeana* and considered it to be a respiration product of this kelp. According to Haldane and Makgill (24), small quantities of carbon monoxide are formed in the oxidation of wet hay at 40° C. if bacteria are excluded. When bacteria were present, they found that hydrogen was formed instead.

In 1941 the Industrial Health Division of the Minnesota Department of Health, employing the Mines Safety Appliances Company carbon monoxide indicator, found up to 0.03 per cent carbon monoxide in the interseed air of sample grade flaxseed in commercial storage.[6] In this apparatus the carbon monoxide is catalytically oxidized to carbon dioxide by means of a special catalyst (hopcalite) and the heat of the reaction measured electrically. Water vapor, hydrogen, and hydrogen sulfide may interfere with the test. Provision is made in the apparatus for the removal of moisture, while hydrogen is only oxidized by the catalyst at elevated temperatures. Qualitative tests for hydrogen sulfide were negative and hence the results obtained with this device provide strong evidence of the actual presence of carbon monoxide in the interseed air of the flaxseed bin.

In connection with the bulk storage experiment described in the previous section, the cooperation of the Minnesota Department of Health was obtained in making analyses for carbon monoxide in gases drawn from various depths in the soybeans. These tests were carried out 62 days after the soybeans were placed in storage. No carbon monoxide was found in the interseed air to a depth of 20 feet below the surface. Several samples drawn from depths of 20 to 50 feet gave carbon monoxide values varying between 0.005 and 0.02 per cent.

Confirmatory evidence of the formation of carbon monoxide was obtained from a sample of soybeans undergoing heating in the small adiabatic respirometer. When the beans attained a temperature of 47° C., gas from the respirometer reduced a saturated solution of palladious chloride. Since the reduction might possibly have been caused by other unknown gaseous decomposition products, a more specific test was carried out by drawing gas from the respirometer through oxalated laked rat blood and testing for the presence of carbon monoxide hemoglobin by the pyrotannic acid method described by Jacobs (27). This test was strongly positive, thus proving the production of carbon monoxide by heating soybeans. The mechanism of its production and the quantities produced under various conditions are still unknown.

[6] Private communication.

DISCUSSION AND CONCLUSIONS

These studies show that moisture content and temperature do not alone determine the respiratory activity of a sample of soybeans. Of very great importance is the history of the sample prior to the time the respiratory rate is determined. Samples at moisture contents above 13 per cent were observed to show increases in respiratory activity as great as several hundred per cent when stored for a few weeks at room temperature. The respiratory rates attained when stored under these conditions bore little relation to the rates found soon after conditioning or after storage at low temperatures.

Several lines of evidence indicate that the high respiratory rates observed after storage at high moisture levels and moderately high temperatures were due chiefly to microorganic activity. Beans which exhibited these high respiratory rates usually were visibly moldy or had a musty or a sour odor and bacteria and a number of fungi were found in abundance. Moreover, higher respiratory rates were observed in nonviable beans than in beans of high viability.

Attempts to sterilize grain and thus separate these two sources of respiration are always likely to affect the respiration of the grain itself; moreover, surface sterilization techniques cannot be effective because bacteria and fungi appear to be present inside as well as on the surface of the soybeans. It is questionable whether the smooth curve given in figure 3 represents the true respiratory activity of soybeans. It can only be stated that the respiration due to microorganisms was at a minimum. While organisms were present, they were not given sufficient time under the proper conditions to proliferate before the respiratory rates of these samples were determined.

That the inherent respiration of soybeans may be relatively unimportant is indicated by the results of the experiment in which heat-treated beans were inoculated with a water extract of moldy soybeans. The respiratory rate of these beans determined after ten days' storage was approximately equivalent to that of unheated beans of the same moisture content stored under similar conditions.

The more vigorous respiration of split than whole soybeans when both were stored under conditions favorable to the development of microorganisms may be explained by the greater surface of the split soybeans on which microorganisms can grow and also by the lack of the seed coat which partially protects whole beans against invasion by microscopic flora.

The fluctuating rates of carbon dioxide production observed in the instance of soybeans undergoing heating in the adiabatic respirometer suggest that different organisms predominate at various stages of the heating process.

The rapid decrease in the viability of soybeans under conditions which favor the growth of microorganisms raises the question whether bacteria and fungi are responsible for the decrease in germination capacity or if they are entirely saprophytic and become active only after the beans are nonviable. This question cannot be answered from the studies conducted but it is clear that conditions which will inhibit the growth of microorganisms are the most favorable for the storage of soybeans.

The lowest moisture content at which soybeans were observed to heat was 15.6 per cent. Increases in respiration with time of storage were marked in soybeans containing over 13 per cent moisture when stored at room temperature; furthermore, the iodine number of oil from such beans was greatly reduced, although the quantity of oil did not seem to be diminished. Soybeans remained sound and retained their viability well at a moisture content of 15.8 per cent for a year and a half when stored at 4° C. On the other hand, when stored at room temperature, viability was seriously diminished even below a moisture content of 10 per cent.

The moistures given above were determined by the two-stage vacuum-oven method. When the equation at the bottom of table 1 is used to convert them into the equivalent Tag-Heppenstall moistures, it is found that as far as moisture content is concerned, soybeans in grade No. 1 may be stored under most conditions; Grade No. 2 may be safe under most conditions of storage but for a shorter time; grades Nos. 3 and 4 involve a great storage risk and should be stored only for short periods at low temperatures. When soybeans are stored at the high moisture contents allowed in these two grades, temperatures should be closely watched and the beans moved when any heating is observed. Damage can occur without excessive heating.

If soybeans are to be kept for seed purposes during warm weather or for a long period of time, it seems advisable to store them at a moisture content of 10 per cent or less. With unfavorable harvest weather, artificial drying may be necessary to reduce the moisture content, and investigations are needed to ascertain the drying conditions which may be safely employed without injury to germination.

If moisture contents before and after kiln-drying are determined by the Tag-Heppenstall moisture meter as it is now calibrated, a large invisible loss is likely to be found. Since this meter gives especially low results on high moisture soybeans, the operator is actually removing more water from the beans than the moisture values indicate.

The biochemical studies carried out with soybeans stored for 12 months at various moisture levels support the view that carbohydrates are the first food materials utilized in respiration. Calculations of the apparent specific heat of soybeans from the data of the adiabatic respirometer experiments also seem to indicate that heating results primarily from the oxidation of carbohydrates. Thus, the apparent specific heats for the data recorded in tables 18 (experiment 3) and 19, computed on the assumption that the carbon dioxide arose entirely from carbohydrates (1 g. $CO_2 = 2.565$ C), gave values of 0.49 and 0.47 small calories per gram dry matter per degree centigrade, respectively; the corresponding values based on the assumption that the carbon dioxide resulted solely from the oxidation of fatty oils (1 g. $CO_2 = 3.408$ C) were 0.75 and 0.71 small calories.[7]

As far as the authors are aware, values for the specific heat of soybeans have not been reported in the literature. While both sets of calculated values are doubtless high, those computed on the basis of carbohydrate oxidation are more in line with what would be expected, as judged from the known specific heats of several semi-drying oils and wheat flour.[8] The available evidence, both from the analyses of soybeans after storage and the results of the adiabatic heating trials, thus indicates that the equation for the oxidation of hexoses given on page 3 may be regarded as representing the quantitative relation between the production of heat and carbon dioxide.

Calculations employing the data obtained in the bulk storage experiment indicate that considerable aeration of soybeans takes place in commercial elevator storage. Thus from table 22 and table 23 it will be noted that after 62 days' storage the temperature of the beans at a depth of 40 feet had increased approximately 30° C. while the carbon dioxide concentration in the interseed air at this level had risen to 16.9 per cent. This temperature

[7] In these experiments strictly adiabatic conditions were not maintained in order to reduce the possibility of heating the soybeans by the surrounding air. The observed temperature increases of the beans were accordingly corrected arbitrarily by adding 0.2° C. per day in making these calculations.

[8] The specific heats of several semi-drying oils compiled in International Critical Tables are in the order of 0.4 calories per gram. Winkler and Geddes (Cereal Chem. 8:455-474, 1931) found that the specific heat of wheat flours averaged 0.397 calories per gram dry matter.

increase would require 1500 calories per 100 g. soybeans, assuming a specific heat of 0.5 and no heat loss. If this heat arose from the oxidation of sugars, 585 mg. carbon dioxide (325 ml. at ordinary temperature and pressure) and 239 mg. water would be produced. The interseed air space, however, amounts to only 46 ml. per 100 g. soybeans of 60 pounds test weight and a specific gravity of 1.2. In other words, for a temperature increase of 30° C., the estimated volume of carbon dioxide produced is approximately six times the volume of the interseed air space. That heating of grain sets up convection currents and results in considerable aeration has recently been indicated by studies carried out by the Division of Agricultural Biochemistry[9] on flaxseed in elevator storage. Samples of interseed air drawn from various depths in sample grade flaxseed of uniform moisture content stored in an interstitial bin exhibited a progressive increase in oxygen and decrease in carbon dioxide at depths below 30 feet.

If all the water produced by respiration in the above calculation were absorbed by the beans, their moisture content would be increased only approximately 0.2 per cent. It therefore appears that any appreciable increase in moisture content in a localized portion of a bin is more likely to be the result of condensation or the hygroscopic uptake of water from atmospheres of high relative humidity than the result of water produced as an end product of respiration.

SUMMARY

Soybeans, within the past few years, have risen from a comparatively obscure place in American agriculture to the rank of a major crop in the central area of the United States. Several factors related to the respiration and keeping qualities of yellow soybeans, which constitute approximately 90 per cent of the domestic production, have been studied.

Moisture contents of soybeans determined by the Tag-Heppenstall moisture meter as it is now calibrated for official grading of soybeans were found to be markedly lower than when determined by a two-stage vacuum-oven method. The Tag-Heppenstall instrument most seriously underestimated the moisture content of high moisture samples; that is, in cases where moisture differences become more critical in relation to respiratory activity and storage behavior. The whole-bean air-oven method of the American Oil Chemists' Society gave results in close agreement with

[9] Unpublished results.

those of the two-stage vacuum-oven method but the differences between duplicates were much greater.

Hygroscopicity of four samples of soybeans representing three varieties at relative humidities of 35, 50, 60, 70, and 85 per cent, averaged 6.5, 8.0, 9.6, 12.4, and 18.4 per cent moisture as determined by the two-stage vacuum-oven method. As compared with published data for the cereal grains, the moisture content of soybeans was found to increase more rapidly with increasing humidity. Soybean oil meals were somewhat more hygroscopic than whole beans; this was attributed to the lower oil content of the former. Toasted solvent-extracted and expeller meals were similar and slightly lower in hygroscopicity than untoasted solvent-extracted meal, but the differences were not great enough to have practical significance. Soybean oil meals were less hygroscopic than wheat flour at relative humidities below 70 per cent but became considerably more hygroscopic than wheat flour at relative humidities above 70 per cent. This implies that such meals will show considerable changes in weight depending on the atmospheric conditions under which they are stored and, furthermore, will be very prone to spoilage when stored in atmospheres of high relative humidities.

Respiration was measured by determining the amount of carbon dioxide produced by a known weight of soybeans during incubation at constant temperature for a four-day period. Except when temperature was the variable studied, this incubation temperature was 37.8° C. (100° F.) since this was regarded as an extreme temperature condition under which grain is likely to be stored. When respiration was measured soon after conditioning beans to a range of moisture levels, the acceleration of respiratory rate with increasing moisture content was very regular, and a smooth exponential curve resulted from plotting moisture content against respiratory rate.

Irregular but very great increases in respiratory rate occurred when samples above 13 per cent moisture content were stored for a few weeks at moderate temperatures before determining respiratory activity. These increases were of the order of magnitude of several hundred per cent and were believed to be caused by the growth of microorganisms. Such samples were visibly moldy or possessed a sour or a musty odor.

Other experiments on the effect of storage temperature, temperature at which respiratory activity was determined, comparative respiration of whole and split soybeans when stored under identical conditions, and the respiration of heat-treated beans which had been dampened with sterile and with inoculated water, all seemed to confirm the theory that microorganisms are the primary cause of the excessively high respiratory rates responsible for heating and other damage to stored soybeans.

Rapid loss of viability was caused by conditions which favored the growth of microorganisms in soybeans. Viability was better retained in beans stored at 15 per cent moisture and a temperature of 4° C. than in beans from the same lot stored at 9 per cent moisture and room temperature. To retain high maximum germination capacity, soybeans should be stored at a low moisture content (approximately 10 per cent) and at as low a temperature as feasible.

Considerable damage was observed in soybeans stored at high moisture levels at room temperature even though heating did not occur. This damage appeared to be caused by microorganisms. Soybeans were stored at a series of moisture contents for a period of one year at room temperature. The most important chemical differences observed were that high moisture beans had more reducing sugar, less nonreducing sugar, and lower iodine number of the oil than those stored at low moisture contents. Catalase activity and *p*H first dropped with increasing moisture but then increased in the case of the moldy samples.

Adiabatic respirometers of two types were used for laboratory studies of soybean heating. The larger held nearly six bushels and was loaded with beans at moisture contents of 18.8, 17.5, 15.6, and 14.7 per cent; all samples heated except the one at 14.7 per cent moisture. Time required to reach maximum temperature decreased with increasing moisture.

The smaller apparatus which held only one quart was used in later experiments. With this equipment, aeration was controlled and carbon dioxide production measured. Soybeans of 24.5 per cent moisture attained a temperature of 88.5° C. Samples placed in the respirometer immediately after conditioning did not heat until after an increase in respiratory rate had occurred; the time required for this increase corresponded very closely with that necessary for the appearance of visible mold growth. Respiratory rate did not show a regular increase with temperature; instead it increased and decreased in irregular waves. This was interpreted as resulting from changes in the predominating flora with changes in temperature. Aeration was found to be necessary for heating to take place.

Bulk storage of 987,000 pounds of soybeans, grading U. S. Nos. 3 and 4 with an average moisture content of 15.82 per cent

(Tag-Heppenstall), for two months resulted in heating despite low atmospheric temperatures. Considerable damage occurred even though the maximum temperature observed was 101° F. Calculations of the theoretical amount of carbon dioxide produced by heating soybeans together with the analyses of gas samples drawn at intervals from various levels in this bin indicated that considerable aeration took place. Moisture produced by respiration even at relatively high rates is not sufficient to increase appreciably the moisture content of soybeans; large localized increases in moisture content are more likely to be the result of condensation or the hygroscopic uptake of water from atmospheres of high relative humidity.

A wide variety of fungi was isolated from soybeans drawn from the bin at the conclusion of the experiment. Bacteria and fungi were found both on the surface and in the interior of the damaged beans.

Carbon monoxide was detected by qualitative tests in the gas drawn from heating soybeans both in the elevator bin and in the small adiabatic respirometer.

The lowest moisture content at which soybeans were observed to heat when stored at laboratory temperature (25°–26° C.) was 15.6 per cent, as determined by the two-stage vacuum-oven method. This corresponds to a moisture content of approximately 14.3 per cent when determined by the Tag-Heppenstall moisture meter as it is now calibrated. Since the permissible moisture limits for grades Nos. 1, 2, 3, and 4 are 13, 14, 16, and 18 per cent, respectively, grades Nos. 3 and 4 involve a great storage risk except for relatively short periods at low temperatures. Subsequent to the completion of these studies, the official basic method for determining the moisture content of soybeans for grading purposes has been changed, and it is anticipated that recalibration of the Tag-Heppenstall moisture meter to the new basis will probably greatly reduce the discrepancy observed in these studies between the moisture values obtained by this instrument and those found by the two-stage vacuum-oven method.

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